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Slowing starch digestibility in foods

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CHAPTER 5

Efficacy of fibre additions to flatbread flour mixes for reducing post-meal glucose and insulin responses in healthy Indian subjects

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ABSTRACT

The incidence of type 2 diabetes (T2DM) is increasing worldwide and also in developing countries, particularly in South Asia. Intakes of foods generating a high post-prandial glucose (PPG) response have been positively associated with T2DM. As part of efforts to identify effective and feasible strategies to reduce the glycaemic impact of carbohydrate-rich staples, we previously found that addition of guar gum (GG) and chickpea flour (CPF) to wheat flour could significantly reduce the PPG response to flatbread products. Based on those results in an exploratory study with Caucasian subjects, we have now tested the effect of additions of specific combinations of CPF with even lower doses of GG to a flatbread flour mix, for their impacts on PPG and insulin (PPI) responses in a South-Asian population. In a randomized, placebo-controlled full-crossover design, 56 healthy Indian adults consumed flatbreads made with a commercial flatbread mix (100% of wheat flour) with no further additions (control) or incorporating 15% CPF in combination with 2, 3 or 4% GG. The flatbreads with CPF and 3 or 4% GG significantly reduced PPG (both $\geq 15\%$ reduction in positive incremental area under the curve, $p < 0.01$) and PPI (both $\geq 28\%$ reduction in total area under the curve, $p < 0.0001$) compared to the flatbreads made with control flour. These results confirm the efficacy and feasibility of the addition of CPF with GG to flatbread flour mixes to achieve significant reductions in both PPG and PPI in Indian subjects.

This trial is registered at [ClinicalTrials.gov](https://clinicaltrials.gov) with identifier NCT 02814357.

INTRODUCTION

There is a growing global epidemic of Type 2 diabetes mellitus (T2DM), especially in developing countries such as India ⁽¹⁾. Consequently there is considerable public health and consumer interest in taking steps to reduce this risk. Continuous exposures to higher PPG and post-prandial insulin (PPI) responses is believed to be detrimental for health, contributing toward an increased risk of (pre-)diabetes ⁽²⁾. There is a wealth of literature showing that reducing PPG (by slowing rates of digestion and reducing bioavailability⁽³⁾) has benefits for reducing the progression from pre-diabetes to T2DM ^(4; 5; 6) and the risk of CVD ^(7; 8).

A lower postprandial insulin requirement may also be beneficial in the short and longer term. Consistent with this, the European Food Safety Authority (EFSA) has recognized that the reduction of postprandial glycaemia may be a beneficial physiological effect, but only if postprandial insulin is not disproportionately increased ⁽⁹⁾.

Because of their frequent and consistent use, carbohydrate-rich staple foods are interesting candidates for reducing PPG and PPI exposures ⁽¹⁰⁾. Wheat-based flatbreads and rice are the two most common carbohydrate-rich staple foods in Southeast Asia ⁽¹¹⁾, making them important contributors to the daily glycaemic load. Flatbreads are usually prepared at home from a commercially-made whole-wheat flour mix ("atta"). Chickpea flour (CPF) and bran-fibre flatbreads are especially advised for subjects with T2DM for whom rice is considered less desirable due to its high glycaemic index (GI) ⁽¹²⁾. Therefore commercially-viable, efficacious routes to further reduce the PPG response to flatbreads are of interest.

Soluble viscous fibres can lower PPG ⁽¹³⁾, by delaying gastric emptying ^(14; 15) and inhibiting the propulsive and mixing effects in the intestine ^(16; 17). In addition, legume flours, such as CPF, are known to give a lower plasma glucose response than wheat flours ⁽¹⁸⁾. Previous research has shown that soluble viscous fibres (viz, beta-glucan, psyllium and fenugreek) with or without legume flour can lower the PPG ⁽¹²⁾; ⁽¹⁹⁾; ⁽²⁰⁾ or PPI ⁽¹²⁾ of flatbreads.

The emphasis of this study was on commercially feasible products. We ⁽²¹⁾ previously selected and tested various additions of guar (GG), konjac or CPF to flatbreads in Caucasian subjects. From that work, the composition with 4% GG in combination with 15% CPF resulted in the largest absolute reduction in PPG and could therefore be considered as a "positive control" for future studies. However, GG is costly and product development work showed it created a poor product with respect to sensory attributes (dough handling, texture, aroma). Lower amounts of GG (2 or 3% GG) in combination with 15% CPF and additions of 3 and 5% (respectively) barley flour (BF) made more acceptable and affordable products⁽²²⁾ and these have been used in this trial, next to the 4% GG and 15% CPF. The reason why there are different amounts of BF included is that higher amounts of GG benefit from more BF to help mask the GG flavour, to

make a more consumer-acceptable product. The present study aims to confirm the efficacy of the combinations with 2 and 4% GG, that were reported in the previous study both with a significant effect, and to further extend this work to the Indian population. The level of added BF contains only small amounts of viscous fibres (≤ 250 mg) of barley β -glucan) and is unlikely to influence efficacy ⁽²³⁾. The control product was a market standard commercial wheat flour-based product (8% dietary fibre), while the test products were based on an alternative commercially available high fibre atta flour (with 5% wheat bran included; 12% dietary fibre). In our previous study we did not see any effect on PPG between the market standard and high fibre atta products ⁽²¹⁾. For the present study the higher fibre atta was deemed a more appropriate (commercially realistic) platform for the further additions of fibre, and these complete formulations were then compared to the usual, 'standard' atta product.

The objectives of this study were to identify one or more flour compositions that give a significant reduction in PPG and PPI after consumption, relative to the market standard product. Exploratory objectives were to estimate the maximum glucose response (C_{max}), the time at which the C_{max} was reached (T_{max}) and slope to C_{max} and glucose and insulin concentrations at 3 hr. As soluble viscous fibres and CPFs are also claimed to increase satiety ^(24; 25) an additional exploratory objective was to assess possible effects on appetite-related and mood parameters. To our knowledge, this is the first study testing such commercially feasible combinations of soluble viscous fibres and legume flour in flatbreads on the combined PPG and PPI response in a Southeast Asian population.

METHODS

Participants

Eighty-seven healthy South-Asian subjects were recruited locally for screening from an existing database of potential participants of Lambda Therapeutics Research Ltd (LTRL, Ahmedabad, India), which executed the study. Subjects who met all the inclusion criteria and had none of the exclusion criteria were considered for participation (see online supporting material (OSM) **Supplemental Table 1**). The study was conducted according to the principles of Good Clinical Practice, the Declaration of Helsinki (2008) and according to applicable local laws and regulations concerning studies conducted on human subjects. Ethical approval was obtained from Ethical Committee Aditya (Ahmedabad, India). Each participant provided written informed consent for the study.

Experimental design

This study used a double-blind, randomized, controlled, full cross-over (within-subject) design. Treatment orders were balanced according to a Williams-type design, and randomized schedule for allocation to treatment orders was generated with SAS software (version 9.2, SAS Institute Inc., USA) by a statistician not involved with subject contact or subsequent data analyses. All subjects involved in the study were blinded as to the nature of the test products. Subjects attended the initial screening day followed by 4 test days, at least 1 week apart. Participants were instructed to minimize changes in their habitual diet and activity during the study period. On the day prior to each test day subjects were instructed to refrain from physical activity and alcohol consumption and to consume a standardized evening meal. All participants fasted overnight (from 20.00 until consumption of the test product) but were allowed to drink water *ad libitum*. Participants were housed at the test centre the evening before the study day. Between 7:30 AM and 10:00 AM in the morning of each test day, subjects consumed four freshly made flatbreads (100 g flour total) with 250 ml water as breakfast, and completed this within a 15 min period at every visit at the same time and day of the week. They were allowed to drink up to 150 ml water every subsequent hour, to be consumed after venous blood drawings and self-reported appetite and mood ratings. The volume of water consumed was registered.

Test product and preparation

The 3 wheat flour-based test products were a total of 100 g (uncooked flour weight) of an existing commercial fibre enriched flatbread high-fibre flour (HFF), (Annapurna, Hindustan Unilever Ltd., India), comprising whole wheat flour plus 5 % bran and the incorporation of 15 g CPF, 2, 3 or 4 g GG and 3 or 5 g BF per 100 g in combinations shown in **Table 1**. A “market standard” Annapurna Atta (100% wheat flour) was used as the control.

All experimental flour mixes were formulated at the pilot plant of Unilever R&D, Bangalore (India) and flatbreads were prepared fresh at the test site. For each single test serving, 100 g flour was kneaded to a soft and uniform consistency with the addition of ~77 ml water and allowed to rest for 30 min, then divided into 4 equal balls of each 40 g and rolled to 2-3 mm thickness. More water was added and absorbed when fibres or legume flour was incorporated (see **Table 1**). Flatbreads were subsequently baked and kept warm until consumption within 30 min of cooking.

Table 1. Composition of Test flatbreads + carbohydrates and dietary fibre in grams and water in w/w%

Composition	Total available carbs (g)	Total dietary fibre (g) AOAC 2009.8	Water w/w%
100 g market standard flour	65	8	37.2
80 g HFF [*] + 15 g CPF ¹ + 2 g GG ² + 3 g BF ³	56	16	40.6
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	54	17	42.2
81 g HFF+ 15 g CPF + 4 g GG	53	18	42.4

*HFF= high fibre flour, CPF =chickpea flour, GG = guar gum and BF= Barley flour

¹Chickpea flour (Avent Agro Pvt. Ltd., Delhi, India)

²Guar gum (Ace Gum Industries PVT. LTD, Mumbai, India); viscosity cold 1% in water, measured by a Brookfield RVF viscometer 20-RPM Spindle no.4, at 30 min: 4500 mpa.s, at 2 hours 5400 CPS and 24 hours: 5500 mpa.s

³BF = barley flour (Cardin Healthcare Pvt. Ltd.Vadodara, Gujrat, India)

Blood collection and glucose and insulin measurements

Venous blood was collected in tubes containing sodium fluoride for plasma glucose analysis and in plain tubes (without any additive) for serum insulin samples. Baseline samples were collected at -15 min (two baseline measurements) prior to the test meals, followed by samples at 15, 30, 45, 60, 90, 120 and 180 min post-prandially. Two consecutive samples were collected at each time point for plasma glucose and serum insulin analyses. All serum samples were centrifuged (192 G-force for 10 min at 4 °C) prior to immediate analysis or storage at -20 °C. Plasma glucose concentrations were measured on VITROS® 5,1 FS, Ortho Clinical Diagnostics, (Johnson & Johnson, USA) (intra (within) day %CV: 0.6% and between (inter) day %CV: 0.7%). Insulin was measured using an immulite 1000® analyzer (Siemens Diagnostics, USA) (intra (within) day %CV: 2.6% and between (inter) day %CV: 4.3%).

Measurement of appetite and mood

Self-ratings of appetite feelings and mood (“are you feeling hungry?”, “do you desire to eat?” and, “are you feeling energetic?” and “are you feeling happy and contented?”) were made at baseline (pre-consumption) and 25, 40, 135 min post-prandially. The questions were asked in the language subjects understood. These were scored on 100 mm Visual Analogue Scales anchored at the low and high end with “not at all” and “extremely” ⁽²⁶⁾.

Statistical Methods

The primary outcome variable was +iAUC_{2hr}, i.e. the area of the PPG response lying above the baseline concentration. A power calculation indicated that a minimum of 44 subjects would be required, to test for the significance of a 40 mmol.min/l difference in PPG +iAUC_{2hr} for each test product relative to the control. This assumed a standard deviation of 50 (based on a previous study in the UK ⁽²¹⁾ adjusted for venous vs

capillary blood), at $\alpha = 0.05$, and $\beta = 0.90$, where α is conserved over the three comparisons with the control by Dunnett's test. Considering the Williams design for 4 treatments and the experience of drop-outs at the test site, 12 additional subjects were included to make the initial sample size $n=56$.

The $+iAUC_{2hr}$ was calculated using the trapezium rule, and linear interpolation was used between time points where the PPG crossed the baseline value to establish the time of crossing. Statistical comparisons were made using a mixed model analysis of variance, with subject as a random effect, product as a fixed effect and baseline (fasting score) as a covariate. The order of product testing, gender and body weight were all included as covariates. Comparisons were only made between the control and other test products and Dunnett's test was used to adjust the multiple comparisons to an overall significance level of 0.05. All analyses were performed with SAS version 9.2 (SAS Institute, Cary, N.C. USA). The secondary variable was $tAUC_{2hr}$ for serum insulin. Exploratory variables included mean value at 3 hr for serum insulin, maximum post-meal plasma glucose concentration (C_{max}), time when this was reached (T_{max}), and mean value at 3 hr for plasma glucose, $tAUC_{2hr}$ glucose and self-reported scores on the 2 appetite- and 2 mood-related measures. The AUC for appetite and mood ratings scales were calculated using the trapezium rule and expressed as the original scale units by dividing by the length of time measured. There were no pre-planned statistical analyses of exploratory measures and therefore only descriptive statistics are presented for these.

RESULTS

Subject baseline characteristics

From an initial eighty-seven subjects screened for participation, 56 subjects were enrolled and 50 subjects (25 male and 25 female) completed the study, with all dropouts occurring prior to the start or after the first test session (**Fig 1**). The baseline characteristics of participants are shown in **Table 2a** and separately by gender in **Table 2b**. Differences between results of the Intention to Treat (ITT) and Per Protocol (PP) analysis were small regarding both effect sizes and statistical significance. However, the PP results are shown and discussed here, because of the fact the current study was a proof of principle study (study of efficacy). For completeness and transparency, the ITT data are available in the online supporting material.

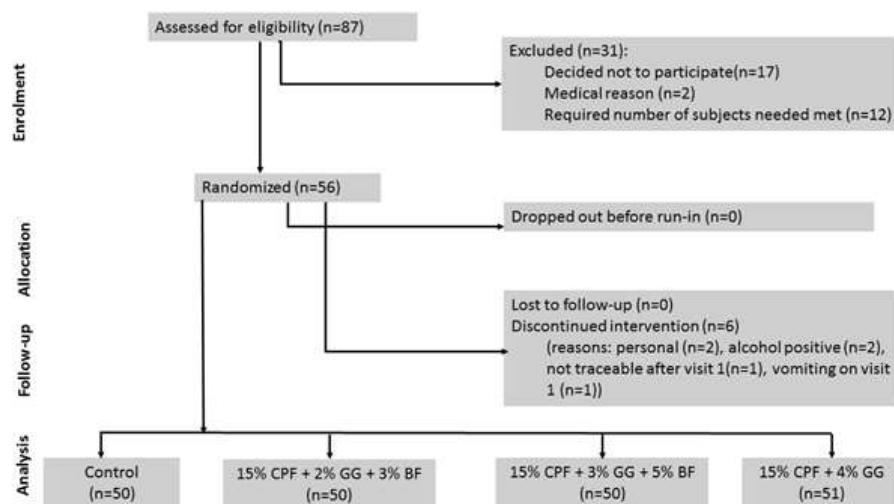


Fig. 1 Flow diagram of participants throughout the study

Table 2a. Subject Baseline Demographic Characteristics (mean and SEM)

	Mean	SEM
Age (yr)	29.16	0.71
Gender (% male)	53.57	
Height (m)	1.59	0.01
Body Weight (Kg)	52.57	0.81
BMI (Kg/m ²)	20.77	0.20
FBG (mmol/l)	4.89	0.03

Table 2b. Subject Baseline Demographic Characteristics by gender (mean and SEM)

	Male (n=30)		Female (n=26)	
	Mean	SEM	Mean	SEM
Age (yr)	27.33	1.05	31.27	0.75
Height (m)	1.66	0.01	1.51	0.01
Body Weight (Kg)	56.21	0.94	48.36	0.76
BMI (Kg/m ²)	20.48	0.28	21.11	0.27
FBG (mmol/l)	4.87	0.05	4.90	0.04

Postprandial plasma glucose concentrations

The PPG response curves are shown in **Fig. 2**, percent differences in plasma glucose +iAUC_{2hr} vs control are shown in **Fig. 4**, and the absolute values and percentage difference given in **Table 3**. All 3 test products resulted in a reduction in the postprandial +iAUC_{2hr}, relative to the control market standard flatbread, and this difference was statistically significant for samples containing 3 and 4% GG (both $p < 0.01$). The data suggest a general dose-response reduction in +iAUC_{2hr} with 2, 3 and 4% GG relative to control; however, this was not tested statistically as no comparisons between treatments were made.

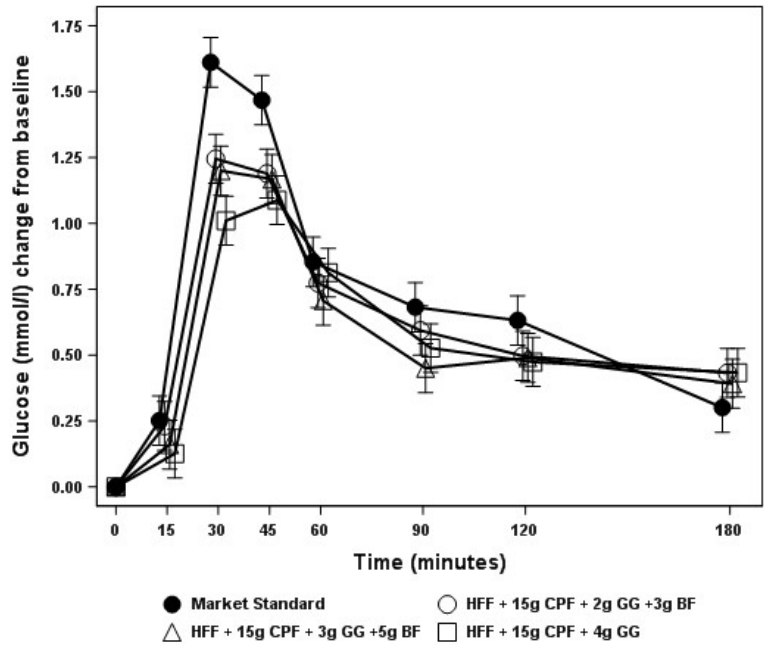


Fig. 2 Effect of flatbread consumption with different amounts of guar gum (2-4%) and 15% chickpea flour on postprandial plasma glucose. (Mean \pm SEM) [HFF = High Fibre Flatbread formulation base]

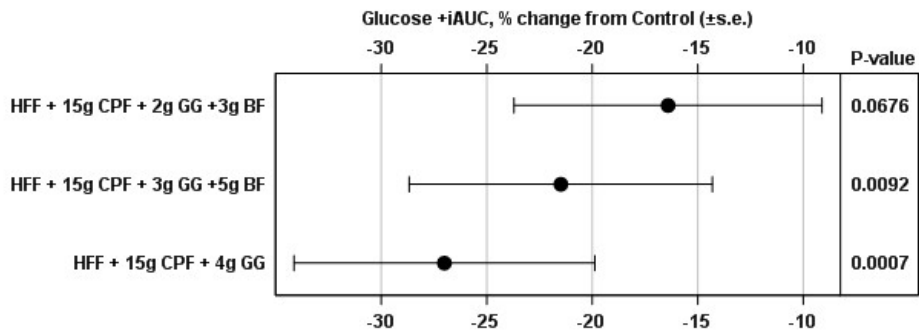


Fig. 4 Percentage change (mean \pm SEM), in PPG (+iAUC_{2hr}) of flatbreads with different amounts of guar gum (2-4%) and 15% chickpea flour and p-value relative to the control

Table 3. Glucose +iAUC_{2hr} (Per protocol data) (mean absolute and [%] difference from control \pm SEM).

Flatbread composition**	Glucose +iAUC _{0-2hr} (absolute and [%] difference from control, 104.9 mmol/l.min)	
	Mean	SEM
80 g HFF + 15 g CPF + 2 g GG + 3 g BF	-17.21 [-16.4%]	7.66 [7.30]
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	-22.54 [-21.5%]*	7.53 [7.18]
81 g HFF + 15 g CPF + 4 g GG	-28.33 [-27.0%]*	7.49 [7.13]

Statistically significant *($p < 0.01$)

* HFF= high fiber flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Postprandial serum insulin concentrations

Data for serum insulin (tAUC) can be found in **Fig. 3**, **Fig. 5** and **Table 4**. All 3 test flatbreads significantly and markedly lowered postprandial total PPI tAUC_{2hr} relative compared to the control flatbread ($p < 0.0001$ for all). In addition, the data suggest a dose-response effect, but this was not tested statistically.

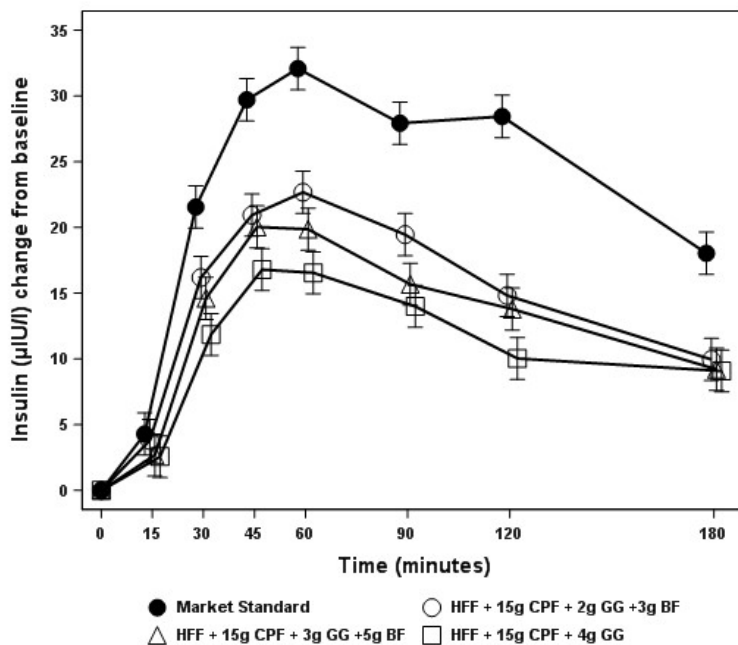


Fig. 3 Effect of flatbread consumption with different amounts of guar gum (2-4%) and 15% chickpea flour on postprandial serum insulin (Mean \pm SEM) [HFF = High Fibre Flatbread formulation base]

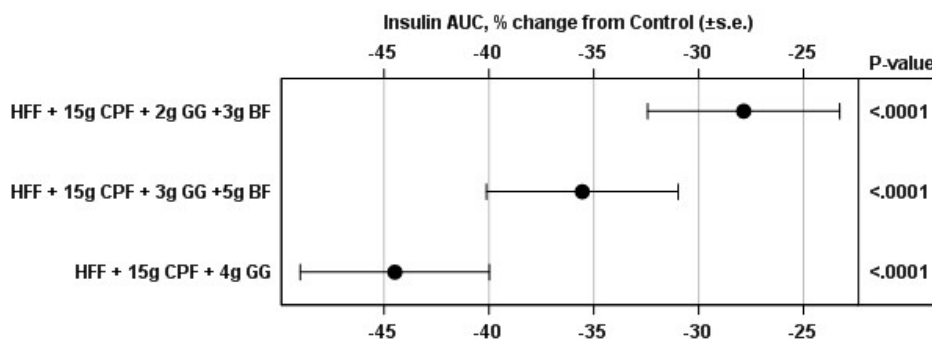


Fig. 5 Percentage change (mean \pm SEM), in PPI ($tAUC_{2hr}$) of flatbreads with different amounts of guar gum (2-4%) and 15% chickpea flour and p-value relative to the control.

Table 4. Insulin tAUC_{0-2hr} (Per Protocol data) (mean absolute and [%] difference from control \pm SEM)

Flatbread composition***	Insulin tAUC _{0-2hr} (absolute and [%] difference from control, 3143.02 μ U/l.min)	
	Mean	SEM
80 g HFF + 15 g CPF + 2 g GG + 3 g BF	-875.42 ** [-27.85]	143.91 [4.58]
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	-1117.30** [-35.55]	143.73 [4.57]
81 g HFF + 15 g CPF + 4 g GG	-1398.20** [-44.48]	141.79 [4.51]

Statistically significant, ** ($p \leq 0.0001$) versus control

*** HFF= high fiber flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Exploratory outcomes

Total AUC (tAUC), C_{max}, T_{max}, 3 hr plasma glucose data and slope to C_{max} data are shown in OSM **Supplemental Table 2a & 2b**. All the treatments resulted in lower mean C_{max} and slope to C_{max} values compared to the control, while T_{max} and plasma glucose at 3 hr were in general little different from the control. Data for serum insulin (+iAUC) can be found in OSM **Supplemental Figure 1**. Serum insulin concentrations at 3hr were consistently lower for all 3 experimental products relative to the control (See OSM **Supplemental Table 3**). No reliable effect of the experimental treatments could be discerned for the appetite or mood measures (See OSM **Supplemental Fig. 2a & 2b** and **Fig. 3a & 3b**).

Adverse Events (AE)

One AE was reported during the conduct of the study. A female subject vomited after consuming the product with 4% GG and 15% CPF, on her first test day. This AE was resolved within 30 minutes without requiring any medical treatment and the subject was excluded from the rest of the study.

DISCUSSION

This study clearly shows that a commercially-feasible, high fibre formulation including the addition of small amounts of GG in combination with CPF lowers the PPG and PPI responses to flatbreads in an Asian population, relative to a commercial “market standard” flour mix. This finding builds on the outcome of our initial exploratory study with Caucasian subjects ⁽²¹⁾ in which four combinations of flour and fibre mixes decreased the +iAUC by more than 30%. From that work the composition with 4% GG and 15% CPF was carried along as “positive control”, because it resulted in the largest absolute reduction in PPG ⁽²¹⁾. However, this amount of GG has adverse impacts on cost, dough handling and aroma, and therefore lower levels of GG were needed. Our research is in line with reports demonstrating that other staple flatbread foods in India (chapattis, naan, rotis) containing dietary fibres and/or legume flours lower the PPG response after a meal ^(12; 19; 20). A novel result is the demonstrated efficacy both on

PPG and PPI reduction of the combination of CPF with lower levels of GG and BF in Asians, as efficacy of this combination previously had been shown only in Caucasians and only on PPG reduction ⁽²¹⁾. This suggests the potential for affordable, efficacious formulations with lower levels of GG.

Although there was a trend for a dose-response effect observed for the additions of 2, 3 and 4% of GG alone on PPG (+iAUC_{2hr}), the effects were statistically significant only for 3 and 4% GG compared to the control product. It seems that addition of 2% GG may be too low to have a consistently meaningful effect related to the effect size on PPG in this food format. Brennan ⁽²⁷⁾ calculated that 2.5% GG lowered the predicted glycaemic index (PGI) in bread by 4%, while 5% GG reduced the PGI by 13% . There was also an indication of a dose-response effect for the Cmax. Other studies have shown the efficacy of GG (ranging from 3.8 g to 14.8 g GG) incorporated into bread for lowering PPG in Caucasians, and these showed a greater reduction in PPG than the outcomes here ^(28; 29; 30; 31).

GG has been found to exhibit viscous characteristics throughout gastric and small intestinal simulation ⁽²⁷⁾, leading to reductions in the rate of gastric emptying and starch digestion and absorption in the intestine, resulting in a lower PPG and insulin response ⁽¹³⁾. GG and some other fibres can even directly inhibit digestive enzymes ^(32; 33). On the product level, viscous fibres can also alter the rheological and/or microstructural properties of the food resulting in reduced ability of the starch to gelatinize during cooking ⁽³⁴⁾. Scanning electron microscopy has shown that guar gum included in bread forms a dense continuous network in which protein, dietary fibre and starch are embedded ⁽²⁷⁾. This dense network was found to be retained after 300 min of *in vitro* digestion, while the majority of the structure of a control bread (without GG) had been degraded ⁽²⁷⁾.

It is possible that the small amounts of added BF might also have influenced the outcomes from this study. However, it appears that 4g of processed barley β -glucan is the minimum required dosage to get a meaningful PPG lowering effect with respect to effect size ⁽²³⁾. The dosages used in this study (3 and 5g) BF (containing 5% [≤ 250 mg] of barley β -glucan) are therefore very unlikely to have meaningfully affected the efficacy.

It is possible that also the addition of CPF contributed to the lower PPG. Replacement of wheat flour by CPF can lower the PPG due to its higher content of resistant starch ⁽³⁵⁾ and high concentration of slowly digestible starch ⁽³⁶⁾. Zafar *et al.* ⁽³⁷⁾ showed that supplementation of whole-wheat bread with at least 35% CPF significantly reduced the glycaemic response of whole-wheat bread, while Johnson *et al.* ⁽³⁸⁾ observed this in white bread.

A lower postprandial insulin requirement may also be beneficial in the short and longer term. In the short term, a lower insulin response prevents hypoglycemia and inappropriate increases of free fatty acids (FFAs) and stress hormone concentrations ^{(39); (40); (41)}, which are often seen during the late postprandial period after consumption of refined carbohydrates ⁽⁴²⁾. Regular consumption of diets with a low PPI response, for example rye-pasta diets, may also benefit individuals with impaired first-phase insulin secretion (first 10-30 min) by allowing β -cell function to recover, leading to improved pancreatic β -cell function in the long-term ⁽⁴³⁾. Higher PPI and fasting insulin concentrations are positively associated with cardiovascular disease (CVD) risk factors such as blood pressure, total cholesterol and LDL-cholesterol ⁽⁴⁴⁾. In addition, insulin resistance and postprandial hyperinsulinemia are related to impaired arterial relaxation, which is an independent predictor of CVD ⁽⁴⁵⁾.

We found that all three test flatbread flour mixes significantly and markedly reduced PPI levels relative to the control. The reason for the lower PPI response of the test flatbreads with fibre and flour mix is probably a slower glucose absorption in the blood, resulting in a reduced stimulation of the entero-insular axis, notably the incretin gastric inhibitory polypeptide (GIP), the secretion of which is directly related to the rate and site of absorption of glucose ^(46; 47; 48). It has also been observed that guar gum in bread directly lowered the GIP response ⁽²⁹⁾. Khawaja ⁽¹²⁾ showed that flatbread containing bran resulted in a lower PPI response than flatbread without bran (19.3 g, 8.6g dietary fibre, respectively) in healthy Asians as well as Asians with T2DM, but our products did contain lower amounts of bran (8 and 12 g for the market standard and high fibre, respectively). Previous reports have demonstrated that supplementation of bread with GG results in a substantially lower PPI ^(28; 29; 30; 49).

The contribution of CPF to the reduced PPI is not clear, as studies on the effect of chickpeas on PPI have yielded mixed results. Nestel *et al.* ⁽³⁶⁾ showed in healthy subjects a serum insulin reduction of 55% at 30 and 60 min after consumption of mashed chickpeas compared to a wheat-based meal ⁽³⁶⁾, while Johnson *et al.* ⁽³⁸⁾ reported an insulin increase of +iAUC of 32% when 24% of the wheat flour was replaced by CPF ⁽³⁸⁾. This discrepancy can be explained by the fact that fine grinding of legumes (as is the case for CPF) disrupts the cell structure and renders starch more readily accessible for digestion ⁽⁵⁰⁾.

No reliable effect on self-reported appetite ratings was apparent for the fibre and CPF mixes used here or in our previous study ⁽²¹⁾. Other research suggests that changes in plasma glucose ^(51; 52) or insulin *per se* ^(52; 53) may have limited effects on appetite. In addition, Clark and Slavin ⁽⁵⁴⁾ concluded in a systematic review that most fibres do not reduce appetite in acute study designs. Nevertheless, there are several reports of enhanced satiety effects associated with the addition of legumes or specific fibres to foods and beverages ^(24; 25). Unfortunately, the levels of viscous fibres needed to

influence appetite may be incompatible with desired sensory attributes of many products⁽⁵⁵⁾.

Comparing the PPG results with those from our previous study ⁽²¹⁾ shows, that the addition of CPF + 4% GG reduced PPG by ~28% in this study, and ~35% in the previous study against slightly different control products; however, the effect here for addition of 2% GG (~16%) was much lower than previously observed (~33%). One of the key differences between these studies was the use of venous blood here, in contrast to capillary blood in the previous study. Venous blood gives a lower PPG response than capillary blood (factor 0.67) ^(56; 57) and this was reflected in the absolute effect size for which the study was powered. The lower PPG values in this study could also be due to a better handling of the glycaemic carbohydrate load in this Indian population (e.g. higher insulin sensitivity), possibly related to the rather low BMI and age range of the subjects. Nevertheless, all the test mixes in the current study gave at least 15% reduction in PPG +iAUC, an effect size which we believe be a reasonable benchmark for a physiologically meaningful effect in a general population. Although this is a subjective judgement, there does not seem to be any threshold for beneficial effects of lowering PPG in reducing CVD risk ⁽⁵⁸⁾. Furthermore, there are intervention studies with the PPG-lowering drug acarbose indicating that this level of reduction is possible and linked to a more effective endogenous insulin secretion effect ⁽⁵⁹⁾.

While this research confirm the effect of specific, commercially-feasible flour/fibre mixes for decreasing the PPG and PPI responses, further research should focus on the mechanism of action (MoA). One of the supposed MoA is a delayed entry of glucose in the systemic circulation originating from starch, largely due to the viscosity generated by GG in the gastrointestinal tract. However, the slow influx of glucose can only be determined by the dual ⁽⁶⁰⁾ or triple stable stable isotope technique ⁽⁶¹⁾. In addition, incretin and glucagon measurements should be included in future studies to better characterise the overall nature of the physiological responses to these kinds of fibre and flour mixes.

Conclusions:

Together with our previous research ⁽²¹⁾, we have demonstrated that flatbread flour mixes incorporating a combination of GG and CPF can produce statistically significant reductions in PPG and PPI responses. The data suggest a dose-response effect at low levels of GG addition, although this would need to be confirmed. However, these additions do not appear to influence post-prandial appetite or mood-related parameters. The results suggest that these additions to commercial flatbread flour mixes could be an efficacious and feasible approach to achieve reduced PPG and PPI responses to such starchy staples in both Caucasian and southeast Asian populations.

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Conflict of interest

All authors are employees of Unilever, which manufactures and markets consumer food products, including the flour used for the flatbreads in this study.

Authorship

HMB, KM, MAMV, PM and DJM designed the research; MAMV and RD facilitated execution of the study; PM performed statistical analysis. HMB wrote the manuscript with significant contributions from DJM, KM, PM and RD. HMB and DJM had primary responsibility for final content. All authors read and approved the final manuscript.

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ONLINE SUPPORTING MATERIAL**Supplemental Table 1:** Criteria for inclusion and exclusion

Inclusion criteria
- Willing to give consent to participate in the study in writing
- Healthy male and female subjects between ≥ 20 and ≤ 50 years of age
- BMI of ≥ 18.0 and ≤ 23.0 kg/m ²
- Apparently healthy: no medical conditions which might affect study measurements as judged by the study physician or measured by questionnaire, and/or assessed by hematology, blood chemistry and urine analysis
- Willing to comply to study protocol during the study
- Agreeing to be informed about medically relevant personal test-results by study physician
- Willing to refrain from drinking alcohol on and one day before the blood withdrawal
- Fasting blood glucose value of volunteer is ≥ 3.4 and ≤ 6.1 mmol/litre (i.e. 62-110 mg/dl) at screening
Exclusion criteria
- Being an employee of Unilever or Lambda Therapeutics Research Ltd
- Chronic smokers, tobacco chewers and drinkers
- Participation in any other biomedical study 3 months before screening visit day of this study and/or participating in any other biomedical study during the screening period
- Intake of too much alcohol (>120 ml/week)
- Reported use of medically prescribed/slimming diet
- Reported participation in night shifts (between 23.00 and 6.00 hrs)
- Use of medication which interferes with study measurements, including vitamins and tonics
- Reported intensive exercise (≥ 10 hrs/week)
- Reported weight loss/gain $\geq 10\%$ of body weight in the 6 months before screening
- Blood donation for 2 months prior to screening
- Blood analysis that showed any drug abuse
- Allergy to any food or cosmetics
- If female, not being pregnant or planning pregnancy during the study period
- If female, lactating or has been lactating for 6 weeks before pre-study investigation and/or during the study period

Supplemental table 2a: Exploratory measures of the glucose response curve (mean absolute and [%] difference from control \pm SEM) (Per Protocol data (PP))

Flatbread composition**	Glucose tAUC _{0-2hr} (absolute and [%] difference from control, 672.83 mmol/l.min)		Glucose Cmax (absolute and [%] difference from control, 6.72 mmol/l)	
	Mean	SEM	Mean	SEM
80 g HFF + 15 g CPF + 2 g GG + 3 g BF	-15.34 [-2.28%]	8.39 [1.25]	-0.38 [-5.66]	0.10 [1.51]
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	-23.47 [-3.49%]	8.25 [1.23]	-0.44 [-6.53]	0.10 [1.49]
81 g HFF + 15 g CPF + 4 g GG	-24.35 [-3.62%]	8.19 [1.22]	-0.56 [-8.31]	0.10 [1.48]

** HFF= high fiber flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Supplemental table 2b: Exploratory measures of the glucose response curve (mean absolute change from control \pm SEM) (PP)

Flatbread composition**	Glucose Tmax (change from control, 44.71 min.)		Glucose at t=3hr (change from control, 5.11 mmol/l)		Slope to C max (change from control, 0.05 mmol/l.min)	
	Mean	SEM	Mean	SEM	Mean	SEM
80 g HFF + 15 g CPF + 2 g GG + 3 g BF	1.08	3.37	0.08	0.07	-0.01	0.003
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	-3.27	3.32	0.07	0.06	-0.01	0.003
81 g HFF + 15 g CPF + 4 g GG	4.52	3.31	0.11	0.06	-0.02	0.003

** HFF= high fiber flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Supplemental table 3: Insulin levels at t=3hr (mean absolute change from control \pm SEM) (PP)

Flatbread composition**	Insulin at t=3hr (change from control, 20.94 μ U/l)	
	Mean	SEM
80 g HFF + 15 g CPF + 2 g GG + 3 g BF	-8.01	1.47
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	-8.06	1.47
81 g HFF + 15 g CPF + 4 g GG	-8.88	1.46

** HFF= high fiber flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Supplemental table 4: Glucose +iAUC_{2hr} (mean absolute and [%] difference from control \pm SEM)) (Intention to Treat Data (ITT)).

Flatbread composition**	Glucose +iAUC _{0-2hr} (absolute and [%] difference from control, 104.8 mmol/l.min)	
	Mean	SEM
80 g HFF + 15 g CPF + 2 g GG + 3 g BF	-17.6 [-16.8]	7.4 [7.10]
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	-23.8 [-22.7%]*	7.4 [7.07]
81 g HFF + 15 g CPF + 4 g GG	-28.4 [-27.1%]*	7.4 [7.09]

Statistically significant *(p<0.01)

** HFF= high fiber flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Supplemental table 5: Insulin tAUC_{0-2hr} (mean absolute and [%] difference from control \pm SEM) (ITT)

Flatbread composition***	Insulin tAUC _{0-2hr} (absolute and [%] difference from control, 3124.5 μ U/l.min)	
	Mean	SEM
80 g HFF + 15 g CPF + 2 g GG + 3 g BF	-882.14. ** [-28.23]	139.77 [4.47]
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	-1142.30** [-36.56]	140.40 [4.49]
81 g HFF + 15 g CPF + 4 g GG	-1377.40** [-44.08]	140.87 [4.51]

Statistically significant: **p<0.0

*** HFF= high fiber flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Supplemental table 6a: Exploratory measures of glucose response curve (mean absolute and [%] difference from control \pm SEM) (ITT)

Flatbread composition**	Glucose tAUC _{0-2hr} (absolute and [%] difference from control, 673.08 mmol/l.min)		Glucose Cmax (absolute and [%] difference from control, 6.74 mmol/l)	
	Mean	SEM	Mean	SEM
80 g HFF + 15 g CPF + 2 g GG + 3 g BF	-15.83 [-2.35%]	8.12 [1.21]	-0.37 [-5.55]	0.10 [1.47]
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	-24.71 [-3.67%]	8.08 [1.20]	-0.47 [-6.98]	0.10 [1.46]
81 g HFF + 15 g CPF + 4 g GG	-24.54 [-3.65%]	8.11 [1.20]	-0.57 [-8.49]	0.10 [1.47]

** HFF= high fiber flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Supplemental table 6b: Exploratory measures of glucose response curve (mean absolute change from control \pm SEM) (ITT)

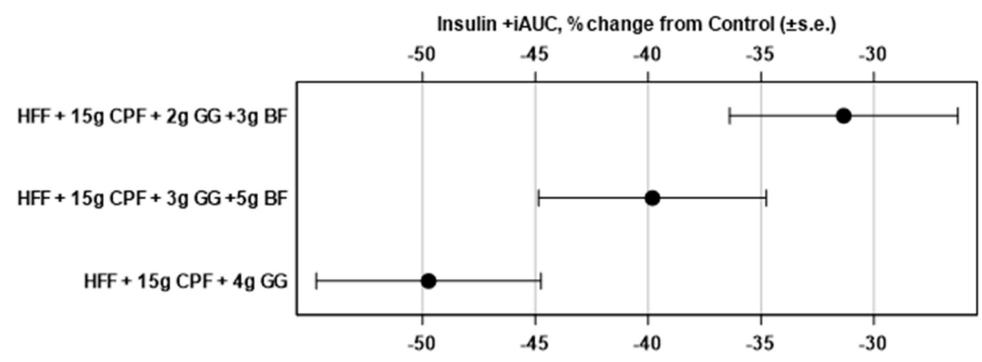
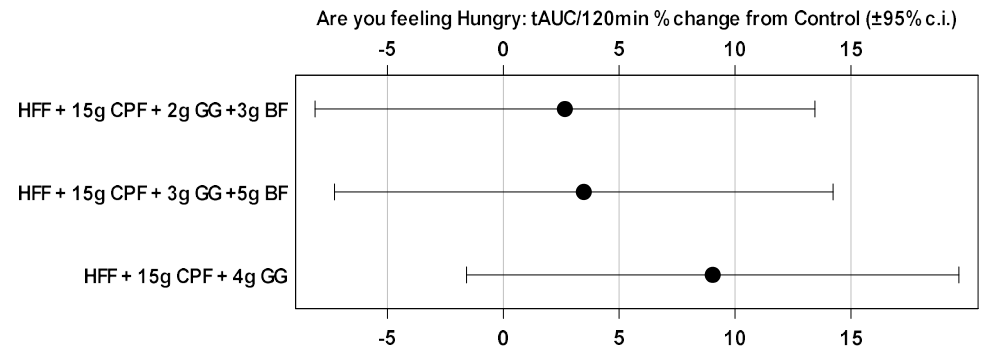
Flatbread composition**	Glucose Tmax (change from control, 44.44min.)		Glucose at t=3hr (change from control, 5.11 mmol/l)		Slope to C max (change from control, 0.050mmol/l.min)	
	Mean	SEM	Mean	SEM	Mean	SEM
80 g HFF + 15 g CPF + 2 g GG + 3 g BF	2.00	3.28	0.08	0.06	-0.012	0.003
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	-2.83	3.26	0.05	0.06	-0.013	0.003
81 g HFF + 15 g CPF + 4 g GG	4.58	3.27	0.11	0.06	-0.018	0.003

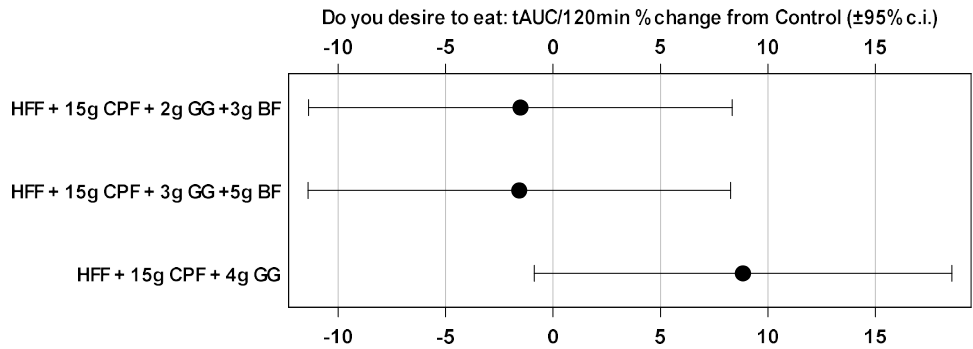
** HFF= high fiber flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Supplemental table 7: Insulin levels at t=3hr (mean absolute change from control \pm SEM) (ITT)

Flatbread composition**	Insulin at t=3hr (change from control, 21.00 μ U/l)	
	Mean	SEM
80 g HFF + 15 g CPF + 2 g GG + 3 g BF	-8.09	1.44
77 g HFF + 15 g CPF + 3 g GG + 5 g BF	-9.23	1.45
81 g HFF + 15 g CPF + 4 g GG	-8.91	1.45

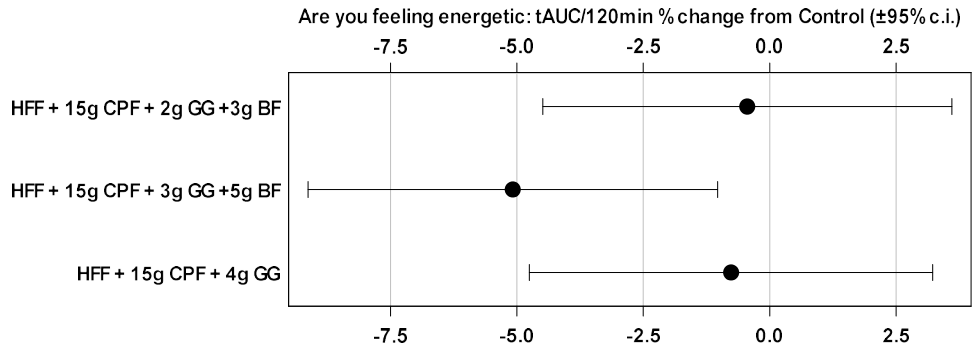
** HFF= high fiber flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

**Supplemental Fig 1.** Percentage change (mean \pm SEM), in insulin(+iAUC_{2hr}) of flatbreads with different amounts of guar gum (2-4%) and 15% chickpea flour relative to the control.**Supplemental Fig 2:** Satiety figures (Per Protocol data)**Supplemental Fig. 2a:** Are you feeling hungry, tAUC/120 min in % change from control (mean \pm 95% C.I.)*



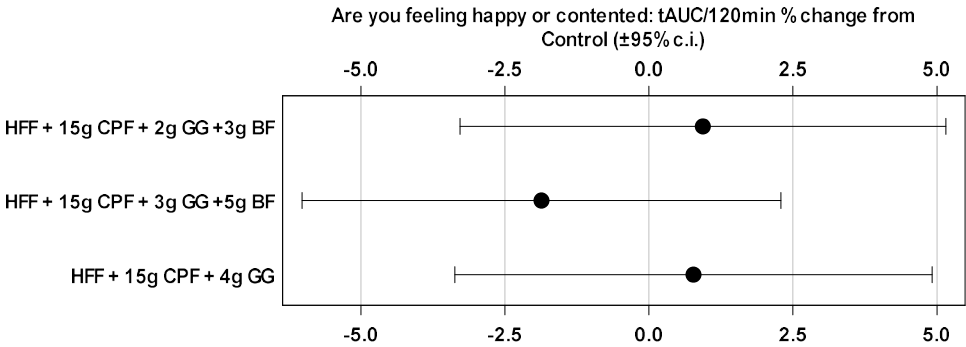
*HFF= high fiber Annapurna flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Supplemental Fig. 2b: Do you desire to eat, tAUC/120 min in % change from control (mean \pm 95% C.I.)*



Supplemental Fig. 3: Mood figures (PP data)

Supplemental Fig. 3a: Are you feeling energetic, tAUC/120 min % change from control (mean \pm 95%



*HFF= high fiber Annapurna flour, CPF =chickpea flour, GG = guar gum and BF = barley flour

Supplemental Fig. 3b: Are you feeling happy or contented, tAUC/120 min % change from control (mean \pm 95% C.I.)*

